2-Keto-D-gluconate dehydrogenase (EC1.1.99.4) from *Gluconobacter melanogenus* is purified according to the procedure of McIntire et al., (McIntire, W., Singer, T.P., Ameyama, M., Adachi, O., Matsushita, K., and Shinagawa, E., Biochem J. (1985) 231, 651 - 654) and references therein. The purified protein is digested with trypsin and chymotrypsin or other proteases to produce peptide fragments which are separated by HPLC or other techniques. Individual peptides are collected and sequenced. From the sequence, DNA probes are synthesized which will anneal to the corresponding sequence in the host organism or a related organism's genome. Using standard PCR techniques, larger fragments of the desired gene are amplified, purified and sequence. These fragments are used to hybridize to the gene and allow for cloning and sequencing of the entire gene. Once the sequence is known, the gene is deleted as described for glucose dehydrogenase (GDH) in Example1.

Other methods to reduce or eliminate 2-keto-D-gluconate dehydrogenase include inhibitors (organic acids such as citrate and succinate are reported to inhibit 2-keto-gluconate dehydrogenase; Shinagawa, E. and Ameyama, M. Methods in Enzymology (1982) 89, 194-198) and changes in pH or temperature.

The enzyme activity can be assayed for activity or loss of activity using the assays described in Shinagawa and Ameyama.

IN THE CLAIMS:

Please cancel claims 1 - 14, 17, 19 and 52 - 57.

Please enter the following clean copy of the amended claims. A marked-up version of the amended claims is submitted on a separate sheet.

15.(Amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order,

a. enzymatically oxidizing the carbon source by at least one oxidative enzymatic activity to an oxidation product wherein said oxidative enzymatic activity requires an oxidized form of an enzymatic co-factor; and

b. enzymatically reducing said oxidation product by at least one reducing enzymatic activity to 2-KLG wherein said reducing enzymatic activity requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between at least one oxidizing step and at least one reducing step and said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and enzymatic derivatives thereof wherein said carbon source is capable of being converted to an ASA intermediate.

18.(Amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

d. enzymatically reducing the third oxidation product by a reducing enzymatic activity to 2-KLG

wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reducing enzymatic activity requires a reduced form of said enzymatic co-factor and wherein the oxidized form and the reduced form of said co-factor are recycled between at least one oxidizing step and the reducing step and said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and enzymatic derivatives thereof wherein said carbon source is capable of being converted to an ASA intermediate .

24.(Amended) The process of Claim 18, wherein each of said first enzyme, said second enzyme and said third enzyme has dehydrogenase activity.

27.(Amended) The process of Claim 25 wherein said second enzyme has GADH activity.

28.(Amended) The process of Claim 25 wherein said third enzyme has KDGDH activity.

29.(Amended) The process of Claim 25 wherein said fourth enzyme is a reductase enzyme.

36.(Amended) The process of Claim 34 wherein said host cell is non-viable.

47.(Amended) The process of Claims 15 and 18 that is continuous.

48.(Amended) The process of Claims 15 and 18 that is batch.

49.(Amended) The process of Claims 15 and 18 that proceeds in an environment comprising organic solvents.

50(Amended) The process of Claims 15 and 18 that proceeds in an environment comprising long polymers.

51.(Amended) The process of Claims 15 and 18 further comprising the step of obtaining ASA from said 2-KLG.

58.(Amended) The process of Claim 15 or Claim 18 wherein said 2-KLG is further purified via electrodiaysis.

Please add the following new claims:

63. A process for the non-fermentative production of 2-KLG from a carbon source comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;

b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;

c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and

d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between the glucose oxidizing step and the reducing step.

64. The process of Claim 63 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

65. The process of Claim 63 wherein said 2,5-DKG reductase is obtainable from a bacterial, yeast or fungal source.

66. The process of Claim 63, that proceeds in an environment comprising exogenously added 2,5-DKG reductase.

67. The process of Claim 63 wherein any one of the dehydrogenases are obtainable from a bacterial, yeast or fungal source.

68. The process of Claim 63 that proceeds in an environment comprising recombinant host cells.

69. The process of Claim 68 wherein said recombinant host cells comprise members of Enterbacteriaces.

70. The process of Claim 69 wherein said recombinant host cell is a Pantoea species.

71. The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous 2,5-DKG reductase.

72. The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous glucose dehydrogenase.

73. A process for the non-fermentative production of 2-KLG from a carbon source comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between the glucose oxidizing step and the reducing step and wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

74. The process of Claim 73 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

75. The process of Claim 74 wherein the host cell is obtained from a Pantoea species and the host cell is modified to eliminate the naturally occurring GDH activity.

76. A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps in any order,

a. enzymatically oxidizing a carbon source selected from the group consisting of glucose, gluconate, and 2-keto-D-gluconate by at least one oxidative enzymatic activity to an oxidation product wherein said oxidative enzymatic activity requires an oxidized form of an enzymatic co-factor; and

b. enzymatically reducing said oxidation product by at least one reducing enzymatic activity to 2-KLG wherein said reducing enzymatic activity requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between at least one oxidizing step and at least one reducing step.

77. The process of Claim 76 wherein the host cells are viable.

78. The process of Claim 76 wherein the host cell is non-viable.

79. The process of Claim 76 wherein the host cell is modified to eliminate the naturally occurring GDH activity and a heterologous GDH having a specificity for $NADP^+$ or NAD^+ is introduced into said host cell.